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REMARKS

The Office action mailed 24 February 2004, has been received and its contents carefully noted. The pending claims, claims 4, 11, 12, 22-25, and 29-31, were rejected. Reconsideration in view of the following remarks is respectfully requested.

Rejection under 35 U.S.C. 102(b)

The Examiner maintained the rejection of the claims under 35 U.S.C. 102(b) as being anticipated by Leishmania Research Project DOD-8b (DOD-8b), Stitler et al. (1994), and Stitler et al. (1995). In the prior Office action, the Examiner deemed that DOD-8b, Stitler et al. (1994), and Stitler et al. (1995) teach a microfluidized lysate preparation.

Applicants respectfully submit that nowhere in the cited prior art is the presence or absence of dextran disclosed. All the cited prior art disclose is that the first generation lysate preparations were reformulated in order to prevent hypersensitivity to preparation and that the reformulated preparations were the subject of an Investigative New Drug Application (IND or INDA) submitted to the U.S. Food and Drug Administration.

It should be noted that many pharmaceutical products containing dextran do not cause hypersensitivity reactions. Also, it should be noted that there is a plethora of agents used in pharmaceuticals which cause hypersensitivity. Thus, one can not extrapolate from the cited prior art that reformulated preparations must be free of dextran.

Additionally, it should be noted that INDs and their contents are confidential and not available to the public.

In order for a reference to anticipate, the reference must be enabling, i.e. teach each and every element of the claimed invention. In the instant case, the cited prior art references do not teach the absence of dextran *specifically* in the reformulated preparations. The present invention as claimed includes the limitation "free of dextran". Clearly, this claimed element is not taught by the cited prior art.

Further, there are several ways to microfluidize a preparation, including freeze thawing and sonication methods known in the art. The present invention as claimed requires that a slurry of at least one Leishmania parasite strain is microfluidized with a sudden release of pressure. Nowhere in the cited prior art is this limitation taught.

Therefore, the prior art does not teach each and every limitation of the invention as claimed. Thus, the rejection under 35 U.S.C. 102(b) should be withdrawn.

The Examiner stated that "the second generation of the lysate was reformulated into a liquid product **(i.e. phenol)** to avoid a suspected hypersensitivity to a component of the lyophilization buffer **(i.e. dextran)**" (emphasis added). From this statement, it appears that the Examiner deems that since the lysate was made into a liquid product, phenol must be present in the formulation and since a component suspected to cause hypersensitivity was removed, the formulation must be free of dextran.

In order to overcome such an assertion by the Examiner, Applicants submit herewith a declaration by Dr. Jonathan B. Berman (Berman Declaration). In the Berman Declaration, Dr. Berman declares that the cited prior art does not provide an enabling disclosure of the present invention as claimed. Specifically, Dr. Berman declares that he has read the cited prior art and does not understand the cited prior art as disclosing the preparations being *free of dextran*, being *microfluidized by a sudden release of pressure*, and *containing phenol*. Dr. Berman also declares that it would not be obvious to him to remove dextran from the formulations in order to prevent hypersensitivity.

In order to anticipate, a reference must be enabling. Since the cited prior art do not provide an enabling disclosure of the present invention as claimed, the cited prior art do not anticipate the present invention. Therefore, the rejection under 35 U.S.C. 102(b) should properly be withdrawn.

In summary, the cited prior art (1) do not teach or disclose each and every element of the claimed invention, and (2) do not provide an enabling disclosure in order to be anticipatory. Thus, the rejection under 35 U.S.C. 102(b) should properly be withdrawn.

Request for an Interview

Should there by any remaining issues after entry of the amendment and consideration of the remarks herein, Applicants respectfully request either an in-person interview or a telephonic interview with the Examiner.

Extension of Time

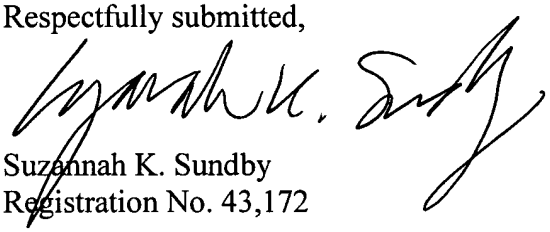
A Petition for an Extension of Time for one (1) month under 37 C.F.R 1.136 and the appropriate fee are submitted herewith to extend the time for responding to the Official Action to 24 June 2004.

CONCLUSION

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. If, however, extensions of time under 37 C.F.R. §1.136 other than those otherwise provided for herewith are required to prevent abandonment of the present patent application, then such extensions of time are hereby petitioned, and any fees therefor are hereby authorized to be charged to our Deposit Account No. 210-380, Attorney Docket No. 034047.013US (WRAIR 98-40/46).

Respectfully submitted,



Suzannah K. Sundby
Registration No. 43,172

Date: 24 June 2004

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Magill, et al.	Group Art Unit: 1645
Serial No.: 09/975,020	Examiner: Shahnan Shah, Khatol S.
Filed: 12 October 2001	Atty Dkt No.: 034047.013US (WRAIR 98-40/46)
For: MICROFLUIDIZED LEISHMANIA LYSATE AND METHODS OF MAKING AND USING THEREOF	

DECLARATION OF JONATHAN J. BERMAN

I, Jonathan J. Berman, reside at 6205 Poindexter Lane, Rockville, MD 20852, declare the following:

1. I have a Ph.D in physics and an M.D. My curriculum vitae is attached.
2. I am the Director, Office of Clinical and Regulatory Affairs, National Center For Complementary and Alternative Medicine of the National Institutes of Health.
3. I have extensive experience in clinical evaluation and drug development with a specialized focus on *Leishmaniasis* and malaria.
4. I have reviewed and understand the Office action mailed 24 February 2004 in the above-referenced application.
5. I have reviewed and understand the pending claims in the above-referenced application.
6. I have reviewed and understand the prior art cited in the Office action, which the cited prior art is:
   a. Leishmania Research project DoD-8B, entitled "Infections *Leishmaniasis* Project Summary". Copy attached.
   b. Stitler et al. (1994) "Good Manufacturing Practices (GMP) Production of *Leishmania* Skin Test Antigen: 1. Protocol Requirements for Investigative New Drug (IND) Application" 44rd Annual Meeting of the American Society of Tropical Medicine and Hygiene. Abstract 179. Copy attached.
   c. Stitler et al. (1994) "Good Manufacturing Practices (GMP) Production of *Leishmania* Skin Test Antigen: 2. Production of a Microfluidized Lysate (MFL) LSTA" 44rd Annual Meeting of the American Society of Tropical Medicine and Hygiene. Abstract 179. Copy attached.
7. Leishmania Research project DoD-8B does not disclose that:
   a. The preparations are free of dextran.
   b. The preparations were microfluidized by a sudden release of pressure.
   c. The preparations contain phenol.

8. Stitler et al. (1994) does not disclose that:

   a. The preparations are free of dextran.
   b. The preparations were microfluidized by a sudden release of pressure.
   c. The preparations contain phenol.

9. Stitler et al. (199s) does not disclose that:

   a. The preparations are free of dextran.
   b. The preparations were microfluidized by a sudden release of pressure.
   c. The preparations contain phenol.

10. Simply reformulating a preparation in order to prevent hypersensitivity does not indicate that the preparation is free of dextran as there are many pharmaceutical preparations that contain dextran but do not cause hypersensitivity.

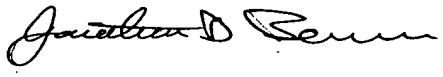
11. The indication that a preparation is a liquid product does not indicate that the preparation contains phenol as there are numerous solutions, solvents, buffers, and pharmaceutical carriers that are used for liquid formulations.

12. There are other ways to microfluidize a preparation which include freeze thawing and sonication. Thus, simply indicating that a preparation is microfluidized does not indicate the specific method by which the preparation was microfluidized.

13. In my opinion, the cited prior art references do not enable one skilled in the art to make and use the microfluidized leishmania lysate preparations of the above-referenced application. Specifically, the cited prior art does not teach microfluidized leishmania lysate preparations free of dextran and microfluidized by a sudden release of pressure. Further, the cited prior art does not teach the use of phenol in the preparations.

14. Further, in my opinion, it would not be obvious to one skilled in the art, such as myself, to remove dextran from the formulations in order to prevent hypersensitivity since there are many pharmaceutical preparations that contain dextran but do not cause hypersensitivity.

15. I declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.



17 June 2004

Ret. Col. Jonathan Berman, MD, Ph.D.

Date

CURRICULUM VITAE--JONATHAN D BERMAN

1. VITAL INFORMATION

EDUCATION:

Jun 1967	B.A., cum laude, High Honors (Chem), Phi Beta Kappa: Williams College
Jan 1972	Ph.D., Biophysics: Harvard University.
Jun 1974	M.D.: Einstein School of Medicine.

2. BOARD CERTIFICATION/TRAINING

Diplomate, American Board of Pediatrics, February 1983.

3. BRIEF CHRONOLOGY OF EMPLOYMENT

1974-1976	Intern and Resident, Pediatrics, Mount Sinai Med Center, N Y.
1976-1977	Infectious Disease Fellow, Cornell Medical Center, New York.
1977-1980	Clinical Associate, Laboratory of Clinical Investigation, Laboratory of Parasitic Disease, NIAID, NIH, Bethesda, MD.
1980-1984	Parasitologist, Division of Experimental Therapeutics (DIV ET), Walter Reed Army Institute of Research (WRAIR) DC.
1984-1988	Clinical Director, Antileishmanial Drug Program, DIV ET
1984-1988	Chief, Biology Department, DIV ET
1988-1989	Assistant Director, Plans and Overseas Operations, WRAIR.
1989-1992	Associate Director, Plans, WRAIR.
1990-1994	Head, AIDS Opportunistic Infections, WRAIR.
1992-2002	Executive Officer, DIV ET
1992-2002	Chief, Biology Department, DIV ET
1999-2002	Research Coordinator: Malaria Drug Discovery and Development
2001-2002	Manager: Severe Malaria Drug Development
July 02 –pres	Dir, Office Clinical and Regulatory Affairs, NCCAM, NIH

4. MILITARY SERVICE

1977-1980	Public Health Service, Bethesda, MD.
1980-2002	U.S. Army Medical Corps-- COL (June 1989)
Aug 2002	Retired after 30 years of total service

5. COMMITTEES

1986-1988	Steering Committee, Leishmaniasis Chemotherapy, TDR/WHO
1991-1994	Ex Officio Member, DAIDS, NIH, Opportunistic Infection Core Committee
1991-1997	Clinical Subcommittee, Integrated Chemotherapy, TDR/WHO
1998- pres	External Product Manager, Miltefosine PDT, TDR/WHO
1998- pres	Chair, CME committee, Am Soc Trop Med Hyg
2002-pres	Chair, Paromomycin PDT, TDR/WHO.

6. RESEARCH INTERESTS

Alternative Med:	Clinical Evaluation
Leishmaniasis:	Biochem Pharmacology/Drug Development/Clinical Investigation
Malaria:	Drug Development / Clinical Investigation

7. IND DIRECTOR (STUDIES SUBMITTED TO US FDA)

DRUG	INDICATION	CO-DEVELOPMENT PARTNER	CLINICAL PHASES
Pentostam	Leishmaniasis RX	Wellcome	
Pre/I/II/III/IV			
Ketoconazole	Leishmaniasis RX	Janssen	II
Paromomycin	Leishmaniasis RX	Teva	Pre/I/II
WR 6026	Leishmaniasis RX	SKB	II
Pentamidine	Leishmaniasis RX	[none]	IV
Azithromycin	Malaria prophylaxis	Pfizer	II/III
WR 6026	P. carinii RX in HIV	NIAID, NIH	I
Azithromycin	M. avium proph in HIV	Pfizer	III

8. MANAGEMENT EXPERIENCE

Organizer/Director of large-scale, multicenter drug trials:

USA:	azithromycin for M. avium
Overseas:	antileishmanial and antimalarial agents

Contact with government/international agencies: FDA, NIH, DoD, WHO

Supervisor of 17-person Department.

Executive Officer for 100-person Division.

Director, Office Clinical and Regulatory Affairs NCCAM, NIH

9. PUBLICATIONS/PRIZES SUMMARIZED

Journal articles:	approximately 100
Review articles:	approximately 15

1997 Louis Weinstein award:Best Infectious Disease article in "Clinical Infectious Diseases" [1997; 24: 686-703]

Editorial Board: Antimicrobial Agents Chemotherapy (1998-2003)

Phi Beta Kappa: Williams College (1967)

"A" Proficiency Designator, USA Medical Corps, Sep 1997

NIH Grant recipient (# UC 1 A149500-01): Azithromcyin combinations for the treatment of P falciparum malaria (Co-PI)

10. MAJOR PUBLICATIONS (by number: R = review)

1) Berman JD, Young DM. Purification and properties of acetylcholinesterase. **Proceedings National Academy Science** USA 1971; 68: 395-398.

R4) Berman JD. Leishmaniasis Chemotherapy: biochemical mechanisms, clinical efficacy, and future strategies. **Reviews Infectious Diseases** 1988; 10: 560-586.

66) Ray P, Berman JD, Middleton W, Brendle J. Botulinum toxin inhibits arachidonic acid release associated with acetylcholine release from PC12 cells. **J Biological Chemistry** 1993; 268:11057-11064.

86) Velez I, Agudelo S, Hendrickx E, Puerta J, Grogl M, Modabber F, Berman J. Inefficacy of Allopurinol for Colombian cutaneous leishmaniasis: a randomized, controlled trial.**Annals Internal Medicine** 1997; 126: 232-236.

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R17) Berman J. Editorial--The FDA approval of AmBisome for the treatment of visceral leishmaniasis. **Clinical Infectious Diseases** 1999; 28: 49-51.

99) Jha TK, Sundar S, Thakur CP, Bachmann P, Karbwang J, Fischer C, Voss A, Berman J. Miltefosine, an oral agent, for the treatment of Indian visceral leishmaniasis. **New England J Medicine** 1999; 341: 1795-1800.

107) Sundar S, Jha TK, Thakur CP, Engel J, Sindermann H, Fischer C, Junge K, Bryceson A, Berman JD. Oral miltefosfine for Indian visceral leishmaniasis. **New England J Medicine** 2002; 347:1739-1746.

R 20) Berman J, Straus S. Research Agenda for Complementary and Alternative Medicines. **Ann Rev Med.** 2004;55:239-254.

R 21) Berman J, Straus S. Complementary and Alternative Medicines for Infectious Diseases. In "**Principles and Practices of Infectious Diseases**" (Mandell GM, Ed). 6th Edition. 2004 (in press)

11. PUBLICATIONS: ALL (major publications denoted by BERMAN)

1) BERMAN JD, Young DM. Purification and properties of acetylcholinesterase. Proc Nat Acad Sci 68: 395-398 (1971).

2) Berman JD. Structural properties of acetylcholinesterase from eel electric tissue and bovine erythrocyte membranes. Biochemistry 12:1710-1715 (1973).

3) Berman JD. How dangerous is penicillin - resistant gonorrhea? Hosp.Physician 13:20 (1977).

4) Berman JD, Johnson WD. Monocyte function in human neonates. Infection and Immunity 19:898-902 (1978).

5) Berman JD, Dwyer DW, Wyler DJ. Multiplication of Leishmania in human macrophages in vitro. Infection and Immunity 26: 375-379 (1979).

6) BERMAN JD, Wyler DJ. An in vitro model for investigation of chemotherapeutic in Leishmaniasis. J Infect Dis 142: 83-86 (1980).

7) Berman JD, Neva FA. Effect of temperature on multiplication of Leishmania amastigotes within human monocyte derived macrophages in vitro. Amer J Trop Med Hyg 30: 318-321 (1981).

8) Berman JD, Dwyer DM. Expression of Leishmania antigen on the surface membrane of infected human macrophages in vitro. Clin Exp Immuno 44: 342-348 (1981).

9) Berman JD. Activity of imidazoles against Leishmania tropica in human macrophage cultures. Am J Trop Med Hyg 30: 566-569 (1981).

10) Berman JD, Beaver PC, Cheever AW, Quindlen EA. Cysticercus of 60-multiliter volume in human brain. Am J Trop Med Hyg 30: 616-619 (1981).

11) Berman JD, Fioretti TB, Dwyer DM. In vivo and in vitro localization of Leishmania within macrophage phagolysosomes: use of colloidal gold as a lysosomal label. J Protozool 28: 239-242 (1981).

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14) Berman JD, Chulay JD, Hendricks LD, Oster CN. Susceptibility of clinically sensitive and resistant Leishmania to pentavalent antimony in vitro. Am J Trop Med Hyg 31: 459-465 (1982).

15) Berman JD, Lee LS. Antileishmanial activity of 8-aminoquinolines in vitro. Am. J. Trop. Med. Hyg. 32: 753-759 (1983).

16) Berman, J.D., and Lee, L.S. Activity of Oral Agents against Leishmania tropica in vitro. Am J Trop Med Hyg 32: 947-951 (1983).

17) Berman JD, Rainey P, Santi DV. Metabolism of formycin B by Leishmania amastigotes in vitro. Comparative Metabolism in infected and uninfected human macrophages. J Exp Med 158: 252-257 (1983).

18) Langreth S, Berman JD, Reardon P, Lee LS. Fine structural alterations in Leishmania tropica exposed to antileishmanial agents in vitro. J Protozool 30: 555-561 (1983).

19) Berman JD, Keenan C, Lamb S, Hanson WL, Waits VB. Leishmania donovani, oral efficacy and toxicity of formycin B in the infected hamster. Exp Parasitology 26: 215-221 (1983).

20) Berman JD, Lee L, Robins RK, Revankar G. Antileishmanial activity of purine analogs against Leishmania tropica within human macrophages in vitro. Antimicrobial Agents Chemotherapy 24: 233-236 (1983).

21) Berman JD, Lee LS. Activity of antileishmanial agents against amastigotes in human monocyte-derived macrophages and in mouse peritoneal macrophages. J Parasitol 70: 220-225 (1984).

22) Berman JD, Oka M, Aikawa M. Fine structural alterations in Trypanosoma rhodesiense grown in vitro, treated with WR 163577. J Protozool 31: 184-186 (1984).

23) Berman JD. Leishmania tropica: quantitation of in vitro activity of antileishmanial agents by Giemsa staining, viability, and 3H-formycin B incorporation. J Parasitol 70: 561-562 (1984).

24) Berman JD, Aikawa M. Activity of immunoglobulin G-coated red cell ghosts containing pentamidine against macrophage-contained Leishmania in vitro. Am J Trop Med Hyg 33: 1112-1118 (1984).

25) Berman JD, Holz GG, Beach OH. Effects of ketoconazole on growth and sterol biosynthesis of Leishmania mexicana promastigotes in culture. Mol Biochem Parasitology 12:1-15 (1984).

26) Nolan LL, Berman JD, Giri L. The effect of formycin B on mRNA translation and uptake of purine precursors in Leishmania mexicana. Biochemistry International 9: 207-218 (1984).

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28) Berman JD, Gallalee J. In vitro antileishmanial activity of IgG-coated red cells containing formycin A. J Infect Dis 151: 698-703 (1985).

29) Berman JD, Waddell D, Hanson BD. Biochemical mechanisms of the antileishmanial activity of sodium stibogluconate. Antimicrobial Agents Chemotherapy 27: 916-920 (1985).

30) Berman JD, Gallalee JV. Semiautomated assessment of in vitro activity of potential antileishmanial drugs. Antimicrobial Agents Chemotherapy 28: 723-726 (1985).

31) Oster CN, Chulay JD, Hendricks LD, Pamplin CL, Ballou WR, Berman JD, Takafuji ET, Tramont EC, Canfield CJ. American cutaneous leishmaniasis: a comparison of three sodium stibogluconate treatment schedules. Am J Trop Med Hyg 34: 856-860 (1985).

32) Berman JD, Gallalee JV, Williams JS, Hockmeyer WD. Activity of pentamidine-containing human red cell ghost against visceral Leishmania in the hamster. Am J Trop Hyg 35: 297-302 (1986).

33) Berman JD, Good LJ, Beach DH, Holz GG. Effects of ketoconazole on sterol biosynthesis by Leishmania mexicana mexicana amastigotes in murine tumor cells. Mol Biochem Parasitology 20: 85-92 (1986).

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37) Berman JD, Hanson WL, Lovelace JK, Waits VB, Jackson JE, Chapman WL, Klein RS. Activity of purine analogs against Leishmania donovani in vivo. Antimicrobial Agents Chemotherapy 31: 111-113 (1987).

38) Berman JD, Gallalee JV, Hansen BD. Uptake of sodium stibogluconate and pentamidine by Leishmania mexicana by macrophages. Experimental Parasitology 64: 127-131 (1987).

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40) Berman JD, Gallalee JV, Best JM, Hill T. Leishmania mexicana amastigotes: uptake, distribution and oxidation of fatty acids. J. Parasitology 73: 555-560 (1987).

41) Ballou WR, McClain JB, Gorden DM, Shanks GD, Andujar J,BERMAN JD, Chulay JD. Safety and efficacy of high-dose sodium stibogluconate therapy of American Cutaneous Leishmaniasis. Lancet 2: 13-16 (1987).

42) Berman JD, Gallalee JV, Gallalee JM. Pharmacokinetics of pentavalent antimony in hamster. Am J Trop Med Hyg 39: 41-45 (1988).

43) Berman JD. Inhibition of leishmanial protein kinase by antileishmania drugs. Am J Trop Med Hyg 38: 138-143 (1988).

44) Berman JD. Antileishmanial activity of red-cell encapsulated drugs. Advances in the Biosciences 67: 145-153 (1987).

45) Murray HW, Berman JD, Wright SD. Synergistic Immunochemotherapy for intracellular Leishmania donovani infection: interferon plus pentavalent antimony. J Infect Dis 157: 973-978 (1988).

46) Berman JD, Grogl M. Leishmania mexicana: Chemistry and biochemistry of Sodium Stibogluconate (Pentostam). Exp Parasitol 1988; 67:96-103.

47) Ray P, Middleton W, Berman JD. Mechanism of agonist induced down-regulation and subsequent recovery of muscarinic acetylcholine receptors in a clonal neuroblastoma-glioma hybrid cell line. J Neurochem 52: 402-409 (1989).

48) Berman JD, Edwards N, King M, Grogl M. Biochemistry of Pentostam-resistant Leishmania. Am J Trop Med Hyg 40: 159-164 (1989).

50) Ray P, Berman JD. Prevention of muscarinic acetylcholine receptor down-regulation by chloroquine: antilysosomal or antimuscarinic mechanisms. Neurochem Res 14: 533-535 (1989).

51) Berman J D, Melby PC, Neva FA. Concentration of Pentostam in human breast milk. Trans Roy Soc Trop Med Hyg 83: 744-745 (1989).

52) Berman JD, King M, Edwards N. Antileishmanial activities of 2,4-diaminoquinazoline putative dihydrofolate reductase inhibitors. Antimicrobial Agents Chemotherapy 33: 1860-1863 (1989).

53) Armijos RX, Chico ME, Cruz ME, Guderian R H, Kreutzer RE, Berman JD, Rogers MD, Grogl M. Human cutaneous leishmaniasis in Ecuador: identification of parasites by enzyme electrophoresis. Am J Trop Med Hyg 43: 424-428 (1990).

54) Saenz R, Paz H, Berman JD. Efficacy of ketoconazole against Leishmania braziliensis panamensis cutaneous leishmaniasis. Amer J Med 89: 147-156 (1990).

55) Franke ED, Wignall FS, Cruz ME, Rosales E, Tovar AA, Lucas CM, Llanos-Cuentas A, BERMAN JD. Efficacy and toxicity of sodium stibogluconate for mucosal leishmaniasis. Annals Int Med 113: 934-940 (1990).

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57) Saenz RE, De Rodriquez CG, Johnson CM, Berman JD. Efficacy and toxicity of Pentostam against Panamanian mucosal leishmaniasis. Amer J Trop Med Hyg. 44: 394-398 (1991) .

58) Guderian RH, Chico ME, Rogers MD, Pattishall KM, Grogl M, Berman JD. Placebo controlled treatment of Ecuadorian cutaneous leishmaniasis. Amer J Trop Med Hyg 45: 92-97 (1991).

59) Ray P, Monroe FL, Berman JD, Fiedler J. Cyanide sensitive and insensitive bioenergetics in a clonal neuroblastoma x glioma hybrid cell line. Neurochemical Research 16: 1121-1124 (1991).

60) Navin TR, Arana BA, Arana FE, Berman JD, Chajon JF. Placebo controlled clinical trial of sodium stibogluconate (Pentostam) versus ketoconazole for treating cutaneous leishmaniasis in Guatemala. J Infect Dis 165: 528-534 (1992)

61) Queener SF, Dean RA, Bartlett MS, Milhous WK, Berman JD, Ellis WY, Smith JW. Efficacy of intermittant dosage of 8-aminoquinolines for therapy or propylaxis of Pneumocystis pneumonia in rats. J Infect Dis 165: 764-768 (1992).

62) Herwaldt BA, Neva FA, Berman JD. Allopurinol in the treatment of American cutaneous leishmaniasis [Letter]. New Eng J Med 327: 498 (1992).

63) Herwaldt BA, Kaye ET, Lepore TJ, Berman JD, Baden HP. Sodium Stibogluconate (Pentostam) overdose during treatment of American cutaneous leishmaniasis. J Infect Dis 165: 968-971 (1992).

64) Berman JD, Ksionski G, Chapman WL, Waits VB, Hanson WL. Activity of amphotericin B Cholesterol dispersion (Amphocil) in experimental visceral leishmaniasis. Antimicrob Agents Chemotherapy 36: 1978-1980 (1992).

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Infections
Leishmaniasis
Project Summary

Title: Development of a Leishmania Skin Test Antigen (LSTA)

Synopsis: This study continues development of a skin test for leishmaniasis (like the skin test for tuberculosis) that would help diagnose this parasitic infection in Gulf War veterans and others who may have been exposed.

Overall Project Objective: Develop an intradermal skin test for the screening of U.S. Service members who may have been exposed to Leishmania parasites during deployments to leishmaniasis endemic areas.

Status/Results to Date: As reported last year, the lyophilized LSTA was reformulated into a liquid product to avoid a suspected hypersensitivity to a component of the lyophilization buffer. A new IND for this reformulated liquid Microfluidized-lysate (MFL)-LSTA was submitted to the FDA in 1999. A Phase I clinical trial was conducted in 15 healthy volunteers which demonstrated safety of the product by showing no significant local or systemic reactions to the product. Additionally, the product was administered in increasing dose and demonstrated that the skin test antigen had no significant local or systemic side effects when used at the planned maximal dose. A RFP was released to identify a commercial manufacturer for the future licensure of the LSTA product. A contract was awarded and phase I/II dose ranging and potency trials are underway.

Project:	DoD-8B
Agency:	Department Of Defense
Location:	Walter Reed Army Institute of Research
P.I. Name:	D. Scott Doughty
Research Type:	Development
Research Focus:	Leishmaniasis
Focus Category:	Infections
Status:	Ongoing
Study Start Date:	October 01,1993
Estimated Completion Date:	January 31,1999

Specific Aims: The goal is to identify a safe, potent, and non-sensitizing Leishmania Skin Test Antigen (LSTA); manufacture it under cGMP; obtain an IND for its use in phase I, II, and III clinical trials; and obtain ultimately a commercially available, FDA-licensed product.

available, FDA-licensed product.

Methodology: Skin tests are widely accepted diagnostic interventions for diagnosis of prior infection with an infectious agent (e.g., tuberculosis). Currently there is no Leishmania skin test licensed for use in the USA. Once required phase I and phase II studies are completed in humans, studies could be performed in Gulf War veterans with confirmed and suspected leishmaniasis.

Most Recent Publications:

None to date.

China University of Medical Sciences, Chengdu, P.R. of China; and General Hospital of Xinjing Petroleum Bureau, Karamay, P.R. of China.

After sequencing the cloned kDNA fragments of the recombinant plasmid pLK 2, we have designed a set of oligomeric DNA primers (I and II) which defined 297 bp kDNA fragments. Dot hybridization analysis revealed it has species specificity. The minimal template kDNA detected is as low as 1 fg, and 2 promastigotes/ml. Amplifying the kDNAs from *Leishmania donovani* Sichuan human isolate, Sichuan canine isolate, *L. infantum*, *L. mexicana*, *L. braziliensis*, *L. major*, lizard *Leishmania*, positive products can be visualized only in *L. donovani* isolates and *L. infantum*. Dot hybridization of the amplified products with pLK2 confirmed that they were *Leishmania* sequences. Based on this set of primers, 8 bone marrow and 4 serum samples from the confirmed visceral leishmaniasis patients were examined, 7 and 2 positive respectively. This result was also confirmed by Southern hybridization. It was shown in experimentally infected golden hamsters that *L. donovani* kDNA could be detected as early as 4 days after infection, so early diagnosis based on detecting kDNA in peripheral blood by PCR amplification is highly promising. Sequence homologies in kDNA of *Leishmania* species causing cutaneous leishmaniasis (CL) in Karamay, Xinjing were analyzed by PCR and kDNA hybridation. Specimens from cutaneous lesions of 8 CL patients (9 samples) were examined by PCR (using primer 13A, 13B), and the amplified products were hybridized with probes of *L. tropica* and *L. gerbilli* separately. Six samples (6/9) showed positive results with *L. tropica* and no hybridation (0/9) occurred with *L. gerbilli*. Southern hybridization was in accordance with those of dot hybridation. Our results suggest that homologous sequences exist within kDNA of *L. tropica* and the species causing CL in Karamay.

179 GOOD MANUFACTURING PRACTICES (GMP) PRODUCTION OF *LEISHMANIA* SKIN TEST ANTIGEN: 1. PROTOCOL REQUIREMENTS FOR INVESTIGATIONAL NEW DRUG (IND) APPLICATION. Stiteler JM*, Ballou WR, Eckels KH, and Magill AJ. Division of Communicable Diseases & Immunology, Walter Reed Army Institute of Research, Washington, DC.

Viscerotropic Leishmaniasis caused by *Leishmania tropica* was described as a new clinical presentation of Leishmaniasis in U.S. troops returning from Operation Desert Storm (ODS). The prevalence of *Leishmania* infection in ODS veterans is unknown. To determine the scope of infection in ODS veterans, a sensitive screening test is needed. One approach is to develop a *Leishmania* Skin Test (LST), which will meet FDA requirements for safety and efficacy. The first step in the development of a safe LST is the production of LST antigen (LSTA) under the strict conditions of the FDA's Good Manufacturing Practices (GMP). Compliance with GMP in production of the LSTA should allow for the approval of Human Use studies with the LSTA by the FDA following their review of an Investi- gational New Drug (IND) Application. Strain WR#1063, which was isolated from a bone marrow aspirate biopsy of a case of viscero- tropic Leishmaniasis was chosen as the type strain of *L.tropica* and source of the LSTA. WR#1063 was cloned, characterized as *L.tropica* by isoenzyme analysis, and then expanded and cryo- preserved as a Master Seed Lot (MSL). One sample of the MSL was expanded under conditions of GMP in WRAIR's Pilot Bioproduction Facility to produce a Production Seed Lot (PSL). Individual samples of the PSL were expanded under GMP to produce Bulk Lot Productions (BLP) of whole promastigotes for use in development of LST protocols for both animal as well as Human Use studies.

180 IDENTIFICATION OF A *TRYPANOSOMA CRUZI* RECOMBINANT ANTIGEN RECOGNIZED BY *T. CRUZI* INFECTED HUMANS AND MICE. Yong TS*, Minning TA, Khimani A, and Dusanic DG. Department of Life Sciences, Indiana State University, Terre Haute, IN.

A *Trypanosoma cruzi* antigen gene with diagnostic potential was identified by screening a Lambda ZAP cDNA library of epimastigote/metacyclic trypomastigotes of *T. cruzi* with laboratory infected BALB/c mice sera. The molecular weight of the fusion protein including β-galactosidase was 34 kDa. Western blots using epimastigote antigen and mice sera immunized with fusion protein showed two bands; 30 kDa and 27 kDa. The recombinant fusion protein reacted strongly with acutely and chronically infected mice and

human sera. Sixteen out of 20 (80%) protein by Western blot or ELISA. S leishmaniasis showed no reactivity recombinant protein. Data from So The insert was about 850 bp in leng

181 DIAGNOSIS OF SYMPTOM THE POLYMERASE CHAI Grogl M, and Berman J. D Research, Washington, DC; India; Federal University o Redwood City, CA; and Be

To diagnose symptomatic viscera aspirates, a polymerase chain rea Leishmania-infected macrophag parasitologically proven kala-aza sensitivity). None of 40 clinically of 13 clinically cured Indian pati (92%). This PCR procedure is ca before therapy, may identify pat substantially obviate the need fc

182 ENZYME POLYMORPH *BRAZILIENSIS*. Kreutz OH.

In a recent report which includ parasites isolated from Southw widely distributed isolates of e study over 200 isolates of *L.* (V to 20 enzymes) have been com species. Few of the enzymes w polymorphism appears to be patients with mucocutaneous frequency comparisons amon Belize), and the MCL enzyme isolates of this New World sp MPI, and 6PGDH.

183 ANTIBODY TO *TRYP* Gabourel I, Bryan J*, Ministry of Health, B the Health Sciences, I

A study was conducted to d disease among three popul Force and from workers on enzyme-linked immunoass radioimmunoprecipitation City Hospital were reactiv

Gushulak B, Gully P, and Blajchman M. Faculty of Medicine - M.D. Programme, McMaster University, Hamilton, ON, Canada; Parasitology, St.Joseph's Hospital and Pathology, McMaster University, Hamilton, ON, Canada; Quarantine Health Services, Health Protection Branch, Health Canada, Ottawa, ON, Canada; and Canadian Red Cross Society and Haematology & Pathology, McMaster University, Hamilton, ON, Canada.

Our goal was to design a culturally acceptable study which will provide a valid estimate of the sero-prevalence of *Trypanosoma cruzi* in Latin-American refugees and immigrants to Canada. A literature search was undertaken to: a) review the scientific research available on *T. cruzi* parasitemia in Canada and the United States, b) explore the current interaction between the Latin-American community in the study area and the Canadian health care system, and c) identify the health programs which are currently in place to service the Latin-American community in the study area. Collaboration with health care workers within the Latin-American community was sought. The implications of the study for the Latin-American community were identified and suitable methods to undertake the study in a culturally-sensitive manner were formulated. We determined a sample size of 450 will be needed to be 95% confident of a sero-prevalence of 5% (plus or minus 2%). These samples will be tested by immunoflourescence or ELISA. A demographic data sheet was developed to stratify participants according to risk factors for antibodies to *T. cruzi*. Barriers to satisfactory interaction of the Latin-American community with the health care system were identified. Recommendations were formulated to ensure the greatest benefit of the study to the Latin-American community. These recommendations addressed the following four issues: 1) community education 2) information dissemination and informed consent 3) follow-up and management. 4) anonymity and confidentiality. printed in Spanish and in Portuguese, as well as English. 3) A clear management plan will be offered to identified participants who test positive for *T. cruzi* including referral to a tropical disease clinic and long-term follow-up. 4) Participants will be given anonymity unless they choose otherwise. All test results will remain confidential.

299 THE DRUG SENSITIVITY PROFILE OF FREE AMASTIGOTES: DEVELOPMENT OF A NEW MODEL SYSTEM FOR SCREENING DRUGS. Grogl M, Portal AC, and Callahan HL. U.S.A. Medical Research Unit-Brazil, Walter Reed Army Institute of Research.

Recently, there have been increasing reports in the literature of at least partially successful *in vitro* culture of "free" amastigotes. Similarly to a drug screen using promastigotes, a drug screen using free amastigotes should be relatively quick and easy, but should be more representative of the situation *in vivo*. In addition, it should alleviate the problems associated with testing drugs against amastigotes in macrophages. We have established an amastigote drug screen using free amastigotes from an L. mexicana (M379) strain as described previously. A comparison of the IC_{50} drug sensitivity profiles of the promastigote and amastigote stages of M379 against reference antileishmanials shows amastigotes and promastigotes respond equally to 3 out of 5 drugs tested. For the other 2 drugs, the IC_{50}s of the free amastigotes are more similar to values found testing amastigotes in macrophages than are the values found testing promastigotes. As expected, amastigotes were more sensitive than promastigotes to all antimony compounds tested (nearly 4-fold to 280-fold depending on the source). A comparison with achievable serum levels *in vivo* (where known) will also be presented.

300 GOOD MANUFACTURING PRACTICES (GMP) PRODUCTION OF *LEISHMANIA* SKIN TEST ANTIGEN (LSTA): 2. PRODUCTION OF A MICROFLUIDIZED LYSATE (MFL) LSTA. Stiteler JM*, Ballou WR, Eckels KH, Wellde BT, Topper MJ, Rowton ED, and Magill AJ. Division of Communicable Diseases & Immunology, Walter Reed Army Instituteof Research, Washington, DC.

Viscerotropic Leishmaniasis (VTL) resulting from infection by *Leishmania tropica* was described as a new clinical presentation of Leishmaniasis following isolation and characterization of the parasite from U.S. troops returning from Operation Desert Storm (ODS). The prevalence of VTL in ODS veterans is unknown. The USA/DoD decided to pursue the development of a LSTA for use as such a diagnostic screening method to determine exposure of personnel to *L. tropica*. A soluble, lyophilized, Microfluidized lysate (MFL) LSTA was developed and produced in accordance with FDA's guidelines for current GMP within WRAIR's Pilot Bioproduction Facility. Strain WR#1063, which was isolated from a bone marrow aspirate biopsy of a case of VTL was chosen as the type strain and source of the MFL-LSTA. WR#1063 was cloned, characterized, and then expanded and cryopreserved (MSL). One sample of the MSL was then expanded (PSL). Individual cryostocks of the PSL of WR#1063 promastigotes were grown, harvested, washed, and stored (BLP). Various BLP processing experiments and animal testing of these LSTA preparations led to the current MFL-LSTA protocol. In brief, the BLP was thawed, microfluidized, centrifuged, the supernatant sterile filtered, the filtrate adjusted to dose, lyophilized as MFL-LSTA. Following required testing of the MFL-LSTA, an INDA was prepared for review by FDA. FDA's approval of human use will lead to Phase I/Phase II trials of the LSTA.